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AD NUMBER
AD834641
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SMUFD, D/A ltr, 15 Feb 1972

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AD834641

TRANSLATION NO. 1847

DATE: 23 September 1966

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JUN 26 1968

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DIAGNOSIS OF TOXIC GASTRO-ENTERITIS DUE TO PATHOGENIC  
COLIBACILLI IN INFANTS BY MEANS OF IMMUNOFLUORESCENCE

Le Concours Medical  
(Medical Concourse)  
Vol 85, 1963, pp 185-188

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The diagnosis of toxic gastro-enteritis in infants due to pathogenic colibacilli often comes up in a very dramatic fashion. The clinician in these cases often examines an infant with a predominant picture of diarrhea and a toxic syndrome. Of course, we know with what frequency diarrhea is encountered in infants in the course of nondigestive disorders. The proof of the digestive etiology of diarrhea is easy to obtain rather quickly if it is due to a parasite or to a digestive insufficiency. But the situation is entirely different when we deal with infectious diarrhea.

Bacteriology of the stool passes its methods on to the conventional bacteriology of isolation and identification. Isolation is obtained on the non-inhibiting environment and on the selective environment, either directly, on the basis of the dilution of the stool or after an enrichment test involving pathogenic germs. Identification is obtained on special environments in "galleries" for the purpose of studying the utilization of the various sugars, proteins, and organic salts. The picture of fermentation activity of the isolated germ is further developed in detail if necessary; this can be done by making a more complete study of its metabolism. This chemical study determines our line of investigation here; the agglutination of the germ by group sera and then by specific sera will confirm the diagnosis and establish the antigenic formula.

As regards the *Escherichia coli* enteropathogens, agglutination may be tried in the best cases after 24 hours and most often after 48 hours; the final diagnosis is not obtained until 2 days later. Thus the methods which are currently used enable us to come up with a diagnosis only 3-4 days later. The clinician cannot be satisfied with this long period of time before he can institute therapy and he has a tendency to demand a bacteriological examination of the stool only as a confirmation of his diagnosis or to support the particular antibiotic selected, as the case may be.

Immunofluorescence, a modern bacteriological method, supplements the conventional methods of biochemical and serological isolation and identification by ~~enabling us to gain~~ valuable time.

The pathological product is spread on the slide (stool scrapings); we agitate a specific fluorescent serum of the ~~germ we are looking for~~ [we are investigating]. If the ~~germs~~ corresponding to the serotype used are present, they appear fluorescent only in the UV microscope in an environment of all the other ~~germs~~ which remain invisible. This is how bacteriological diagnosis by immunofluorescence is schematized to the extreme. The orientation diagnosis is obtained within less than 2 hours.

Immunofluorescence is a very general method which has a very promising future; it dates back to 1941 and Coons who managed to mark an antibody with a fluorescent substance and show its specificity. After that numerous authors studied this problem, including: Beutner, Greech, Thomson, Noirm. In France, the outstanding work of Le Minor, Fournier and Eliachar, as well as the thesis of Fournier in 1962 gave us documents of the utmost interest with respect to the utilization of the method for the diagnosis of enteropathogenic colibacilli.

Today we can selectively [electively] make a germ fluorescent with the help of 2 methods whose description we want to simplify as much as possible here.

In the direct method, the antigenic microbe is placed in contact with a specific antibody serum which is coupled with a fluorescent substance. The antigenic microbe becomes fluorescent because it is covered with fluorescent antibodies. This method involves the inconvenience of having to mark each of the specific sera with a fluorescent substance; this means that we must have a very complete range of marked sera. Now, the operation of coupling antibodies and fluorochrome is relatively troublesome and delicate; the multiplication of these operations naturally increases the risk of denaturing a serum and this of course also increases the risk of error.

In the indirect method, the antigen microbe is always placed in contact with a specific antibody serum but the latter is not fluorescent. It covers the antigenic microbe but it remains invisible in fluorescence microscopy. To make the pair consisting of the antigenic microbe and the specific antibody, fixed on it, fluorescent, we take recourse to a little trick which consists in fixing another antibody, which itself is fluorescent, to the cover [coating] of the nonfluorescent antibody.

If the first specific antibody of the nonfluorescent germ is prepared on rabbits, for example, we can use an antibody which is this time a specific one, in this case a specific antibody of rabbit globulins, marked by a fluorescent substance. Most often we use a fluorescent serum of goat [and] rabbit antiglobulin. Thus the antigenic microbe, on which we have fixed a first [initial] specific antibody of the germ, becomes visible under

the fluorescence microscope when we have fixed on it a second specific fluorescent antibody of the animal species which provided the first antibody. The advantage of this method resides in the fact that we need to prepare only one anti-rabbit or anti-human fluorescent antibody for each case [sample] and this is valid for all of the specific antibodies of germs. The most frequently used fluorescent body is isothiocyanate of fluoresceine (Prolabo); it is used most frequently because it is easily obtained and because it denatures the proteins, on which we fix it, only very little. Its UV fluorescence is green.

The material [used] in fluorescence microscopy involves a very powerful source of UV light, a mercury vapor lamp with quartz of the type H 80-200 Watts Osram, with emission filters, as well as with an anticaloric filter. The microscope is equipped with an ultra-microscopic device with a black background, immersion objectives with diaphragm and safety arrest filters, eliminating the UV rays.

We have been using the direct method in a systematic fashion on the stool of children sent in for coproculture for one year.

Our statistics however are not sufficient to make up an exhaustive study; nevertheless we can make a few statements as to the usefulness of these reactions.

With the serum lots which we used, it seems that a distinction can be made from the very beginning between the group of *Escherichia coli* 026, B6, 055 B5, 0111 B4 and the other enteropathogenic *Escherichia coli*.

In the first group the presence of fluorescent bacilli in the preparation always corresponded to the discovery of enteropathogenic *Escherichia coli* by means of the conventional methods.

In the second group, on the other hand, we often found a few fluorescent bacilli on the preparations and the conventional methods did not always bring out the enteropathogenic *Escherichia coli*.

It is difficult to blame the insufficiency of the conventional techniques in all of these cases, especially since this might only be due to the frequency of this finding.

It would appear to be a better idea to take a closer look at the role of the antigenic relationships or the absence [shortage] of serum specificity; this absence of specificity could be tied in either with an insufficient absorption of the parasite antibodies or with a denaturation of the antibody proteins during the marking manipulation.

After we eliminate these cases, the presence of enteropathogenic *Escherichia coli* in a stool sample may manifest itself in two different ways.

Either we can see thousands of fluorescent bacilli in each microscopic field; on the clinical level, this always then involves acute coli bacilli gastro-enteritis. Using the conventional methods, we learn that the enteropathogenic *Escherichia coli* [*Escherichia coli* enteropathogens] constitute 80-100% of the cultivable coli bacilli in the stool.

Or we only find a few fluorescent germs scattered in the preparation. The interpretation of the immunofluorescence [results] is then a very delicate thing and only the conventional techniques, applied to a large number of colonies (20 is the minimum here) would enable us to come up with a reliable diagnosis of the germ carrier.

Either way, when we find a very large number of bacilli -- in the stool of an infant -- which has been rendered fluorescent by a marked anti-*Escherichia coli* enteropathogen serum, we are correct in strongly assuming infantile gastro-enteritis coli bacilli diarrhea. This diagnosis involves of course a suspicion; when passed on to the clinician, it makes it possible to initiate the appropriate treatment immediately. The laboratory can then more calmly make a conventional study by means of agglutination and, in particular, it can go into a very calm search for O antigens; this search is indispensable for the final diagnosis; we use this only when the [results of the] conventional techniques cannot be obtained for a very long time.

Finally, in those cases where we did not have any fluorescent bacillus on the slide, we were never able to evidence... [page 187 of photostat missing]

...of *Escherichia coli* enteropathogen and the diagnosis can now apparently be discarded.

Immunofluorescence is easy to apply and gives us a diagnosis of a bacterial kind [species] which is sufficiently precise to be used in the clinic. Here is another fact that is of the utmost importance: we can practically and reliably eliminate a diagnosis of toxic gastro-enteritis with pathogenic colibacillus if the test is negative.

Within a few hours only, we can make a rapid epidemiological investigation of the surroundings of the patient and we can make a systematic check on all of the patients or germ carriers.

The study of routine case histories which has been going on for 1 year now enables us fully to confirm the conclusions of the authors who spelled out the specificity and sensitivity of the method. Whitaker, Nelson, Cohen, Thomason, Le Minor find excellent correlation between immunofluorescence and the agglutination method.

The method of bacteriological diagnosis by means of immunofluorescence may become a part of the routine practice of the medical biology laboratories. The equipment has now been perfected and the sera and

fluorochromes can easily be obtained. Immunofluorescence is a general method and we know all about the use to which it has already been put in the diagnosis of syphilis. In the immediate future, we can look forward to using this method in investigating the bacillus of whooping cough in rhino-pharyngeal exsudate, the gonococcus in genital discharge, particularly in women, the pathogenic staphylococcus in the stool, the group A streptococcus in the throat, etc.

All of these research operations are frequently long and sometimes difficult when we use the conventional bacteriological techniques. These techniques however should always be applied in order to verify, control, and spell out more precisely the rapid diagnosis obtained by means of immunofluorescence.

Immunofluorescence is a very reliable and rapid method and is very important as such in emergency diagnoses in toxic gastro-enteritis in infants due to pathogenic coli bacilli. The help which this method can give us should make it mandatory in all bacteriological examinations of the stool of infants.

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